AMENDMENTS TO THE SPECIFICATION

Please replace paragraph [0014] with the following amended paragraph:

The above-mentioned method and compositions of the present invention have the advantages of eliciting a systemic, non-antigen specific immune response in a mammal, and more particularly, of eliciting a systemic, anti-viral immune response in a mammal. Additionally, the method and composition of the present invention can elicit a systemic, anti-tumor immune response in a mammal. Such an anti-tumor immune response can result in the reduction of a tumor in the mammal. The method and composition of the present invention can also elicit a systemic, protective immune response against allergic inflammation in a mammal. The systemic, non-antigen-specific immune response elicited by the method and composition of the present invention result in an increase in effector cell activity, and particularly, natural killer (NK) cell activity in the mammal, and additionally can result in increased production of [[IFN*]] IFN-γ in the mammal.

Please replace paragraph [0025] with the following amended paragraph:

Yet another embodiment of the present invention relates to a method to elicit a systemic, non-specific immune response in a mammal, which includes administering to the mammal a therapeutic composition by a route of administration selected from intravenous and intraperitoneal, wherein the therapeutic composition comprises: (a) a liposome delivery vehicle; and, (b) a recombinant nucleic acid molecule comprising an isolated nucleic acid sequence encoding a cytokine, the nucleic acid sequence being operatively linked to a transcription control sequence. The method of the present invention is particularly useful for eliciting a systemic, anti-viral immune response or a systemic; an anti-tumor immune response; a systemic, protective immune response against allergic inflammation in the mammal; and/or for reduction of a tumor in the mammal. Additionally, the method increases production of [[IFN*]] IFN-γ in the mammal and/or increases natural killer (NK) cell activity in the mammal. In one embodiment, the route of administration is intravenous. The cytokine can include hematopoietic growth factors, interleukins, interferons, immunoglobulin superfamily molecules, tumor necrosis factor family molecules and/or chemokines. In one embodiment, the cytokine is an interleukin, and in a more preferred embodiment, the interleukin is selected from the group of interleukin-2 (IL-2), interleukin-7 (IL-7), interleukin-12 (IL-12), interleukin-15 (IL-15), interleukin-18 (IL-18) or ~~interferon *~~ interferon-γ ~~(IFN*)~~ (IFN-γ), and in an even more preferred embodiment, the interleukin is

selected from the group of interleukin-2 (IL-2), interleukin-12 (IL-12), interleukin-18 (IL-18) or ~~interferon-*~~ interferon-γ ~~(IFN*)~~ (IFN-γ).

Please replace paragraph [0029] with the following amended paragraph:

In one embodiment, any of the above compositions of the present invention administered to a mammal by the present methods can include a recombinant nucleic acid molecule having a nucleic acid sequence encoding a cytokine. In this embodiment, the nucleic acid sequence encoding a cytokine is operatively linked to a transcription control sequence. In the compositions which include a nucleic acid sequence encoding an immunogen, the nucleic acid sequence encoding a cytokine can be in the same or separate recombinant nucleic acid molecule which contains the nucleic acid sequence encoding the immunogen. The nucleic acid sequence encoding a cytokine and the nucleic acid sequence encoding an immunogen can be operatively linked to the same or different transcription control sequences. In preferred embodiments, the cytokine is selected from the group of hematopoietic growth factors, interleukins, interferons, immunoglobulin superfamily molecules, tumor necrosis factor family molecules and/or chemokines. In one embodiment, the cytokine is an interleukin, and in a more preferred embodiment, the interleukin is selected from the group of interleukin-2 (IL-2), interleukin-7 (IL-7), interleukin-12 (IL-12), interleukin-15 (IL-15), interleukin-18 (IL-18) or ~~interferon-*~~ interferon-γ ~~(IFN*)~~ (IFN-γ), and in an even more preferred embodiment, the interleukin is selected from the group of interleukin-2 (IL-2), interleukin-12 (IL-12), interleukin-18 (IL-18) or ~~interferon-*~~ interferon-γ ~~(IFN*)~~ (IFN-γ).

Please replace paragraph [0042] with the following amended paragraph:

Figure 8 is a bar graph illustrating that [[IFN*]] IFN-γ release by immune cells is induced by administration of CLDC, but not lipid or DNA alone.

Please replace paragraph [0043] with the following amended paragraph:

Figure 9 is a bar graph showing that administration of CLDC, but not poly I/C or LPS, induces [[IFN*]] IFN-γ production by splenocytes *in vivo*.

Please replace paragraph [0044] with the following amended paragraph:

Figure 10A is a bar graph showing that NK cells are the source of [[IFN*]] IFN-γ production in splenocytes elicited by intravenous administration of CLDC injection.

Please replace paragraph [0045] with the following amended paragraph:

Figure 10B is a bar graph showing that NK cells are the source of [[IFN*]] IFN-γ production in lung mononuclear cells elicited by intravenous administration of CLDC injection.

Please replace paragraph [0058] with the following amended paragraph:

Figure 17C is a bar graph illustrating that intravenous administration of CLDC encoding [[IFN*]] IFN-γ induces intrapulmonary [[IFN*]] IFN-γ expression.

Please replace paragraph [0070] with the following amended paragraph:

Figure 23 is a bar graph showing that CLDC-mediated immunization with a tumor antigen induces antigen-specific production of [[IFN*]] IFN-γ by splenocytes.

Please replace paragraph [0074] with the following amended paragraph:

Figure 27 is a line graph illustrating that intravenous pulmonary transfection with CLDC containing DNA encoding [[IFN*]] IFN-γ inhibits the development of airway hyperresponsiveness in allergen sensitized and challenged mice.

Please replace paragraph [0075] with the following amended paragraph:

Figure 28 is a bar graph demonstrating that intravenous pulmonary transfection with CLDC containing DNA encoding [[IFN*]] IFN-γ inhibits eosinophil influx to the airways in mice sensitized and challenged with allergen.

Please replace paragraph [0076] with the following amended paragraph:

Figure 29A is a bar graph illustrating that intravenous administration of CLDC induces [[IFN*]] IFN-γ release from spleen as compared to intratracheal administration.

Please replace paragraph [0077] with the following amended paragraph:

Figure 29B is a bar graph illustrating that intravenous administration of CLDC induces [[IFN*]] IFN-γ release from lung as compared to intratracheal administration.

Please replace paragraph [0089] with the following amended paragraph:

One embodiment of the present invention is a method to elicit a systemic,

non-antigen-specific immune response in a mammal ~~immune response in a mammal~~. In this method, a therapeutic composition which includes: (a) a liposome delivery vehicle; and (b) an isolated nucleic acid molecule that is not operatively linked to a transcription control sequence, is administered by intravenous or intraperitoneal administration to a mammal. Administration of such a composition by the method of the present invention results in the elicitation of a systemic, non-antigen-specific immune response in the mammal to which the composition is administered. As discussed above, this immune response additionally has strong, systemic, anti-tumor, anti-allergic inflammation (i.e., protective), and anti-viral properties. Such properties include the activation of NK cells (as measured by upregulation of NK cell markers, such as NK1.1, for example, or by production of [[IFN*]] IFN-γ, production of Th1-type cytokines (e.g., [[IFN*]] IFN-γ) and the non-antigen-specific recruitment and upregulation of activity in mononuclear cells and T lymphocytes.

Please replace paragraph [0095] with the following amended paragraph:

Elicitation of an immune response in a mammal can be an effective treatment for a wide variety of medical disorders, and in particular, for cancer, allergic inflammation and/or infectious disease. As used herein, the term "elicit" can be used interchangeably with the terms "activate", "stimulate", "generate" or "upregulate". According to the present invention, "eliciting an immune response" in a mammal refers to specifically controlling or influencing the activity of the immune response, and can include activating an immune response, upregulating an immune response, enhancing an immune response and/or altering an immune response (such as by eliciting a type of immune response which in turn changes the prevalent type of immune response in a mammal from one which is harmful or ineffective to one which is beneficial or protective. For example, elicitation of a Th1-type response in a mammal that is undergoing a Th2-type response, or vice versa, may change the overall effect of the immune response from harmful to beneficial. Eliciting an immune response which alters the overall immune response in a mammal can be particularly effective in the treatment of allergic inflammation, mycobacterial infections, or parasitic infections. According to the present invention, a disease characterized by a Th2-type immune response (alternatively referred to as a Th2 immune response), can be characterized as a disease which is associated with the predominant activation of a subset of helper T lymphocytes known in the art as Th2-type T lymphocytes (or Th2 lymphocytes), as compared to the activation of Th1-type T lymphocytes (or Th1 lymphocytes). According to the present invention, Th2-type T lymphocytes can be characterized by their production of one or more cytokines, collectively

known as Th2-type cytokines. As used herein, Th2-type cytokines include interleukin-4 (IL-4), interleukin-5 (IL-5), interleukin-6 (IL-6), interleukin-9 (IL-9), interleukin-10 (IL-10), interleukin-13 (IL-13) and interleukin-15 (IL-15). In contrast, Th1-type lymphocytes produce cytokines which include IL-2 and [[IFN*]] IFN-γ. Alternatively, a Th2-type immune response can sometimes be characterized by the predominant production of antibody isotypes which include IgG1 (the approximate human equivalent of which is IgG4) and IgE, whereas a Th1-type immune response can sometimes be characterized by the production of an IgG2a or an IgG3 antibody isotype (the approximate human equivalent of which is IgG1, IgG2 or IgG3).

Please replace paragraph [0097] with the following amended paragraph:

According to the present invention, elicitation of a non-antigen-specific immune response (i.e., a non-specific immune response) includes stimulation of non-specific immune cells, such as macrophages and neutrophils, as well as induction of cytokine production, particularly [[IFN*]] IFN-γ production, and non-antigen-specific activation of effector cells such as NK cells, B lymphocytes and/or T lymphocytes. More specifically, the systemic, non-antigen-specific immune response elicited by the method and composition of the present invention result in an increase in natural killer (NK) cell function and number in the mammal, wherein an increase in NK function is defined as any detectable increase in the level of NK cell function compared to NK cell function in mammals not immunized with a composition of the present invention, or in mammals immunized with a composition of the present invention by a non-systemic (i.e., non-intravenous, non-intraperitoneal) route of administration, with the amount of nucleic acid delivered and the ratio of nucleic acid:lipid being equal. NK function (i.e., activity) can be measured by cytotoxicity assays against a suitable target cell. An example of a suitable target cell by which to measure NK cell cytotoxic activity is YAC-1. An example of an NK cell cytotoxicity assay is presented in Example 1 (Figure 11). NK cell activation can be measured by determining an upregulation of NK1.1/CD69 on cells in various organs, including spleen, lymph node, lung and liver, by flow cytometric analysis (See Example 1, Figs. 1 and 2). Additionally, the systemic, non-antigen-specific immune response elicited by the method and composition of the present invention can result in an increase in production of [[IFN*]] IFN-γ by the NK cells in the mammal in various organs including spleen and lung, wherein an increase in [[IFN*]] IFN-γ production is defined as any detectable increase in the level of [[IFN*]] IFN-γ production compared to [[IFN*]] IFN-γ production by NK cells in mammals not administered with a

composition of the present invention, or in mammals administered with a composition of the present invention by a non-systemic route of administration, with the amount of nucleic acid delivered and the ratio of nucleic acid:lipid being equal. [[IFN*]] IFN-γ production can be measured by a [[IFN*]] IFN-γ ELISA (as is known in the art; Example 1, Figure 10). Preferably, a composition of the present invention administered by the method of the present invention elicits at least about 100 pg/ml of [[IFN*]] IFN-γ per 5 x 106 mononuclear cells from blood, spleen or lung, and more preferably, at least about 500 pg/ml of [[IFN*]] IFN-γ, and more preferably at least about 1000 pg/ml of [[IFN*]] IFN-γ, and even more preferably, at least about 5000 pg/ml of [[IFN*]] IFN-γ, and even more preferably, at least about 10,000 pg/ml of [[IFN*]] IFN-γ.

Please replace paragraph [00116] with the following amended paragraph:

A preferred cytokine nucleic acid molecule of the present invention encodes a hematopoietic growth factor, an interleukin, an interferon, an immunoglobulin superfamily molecule, a tumor necrosis factor family molecule and/or a chemokine (i.e., a protein that regulates the migration and activation of cells, particularly phagocytic cells). A more preferred cytokine nucleic acid molecule of the present invention encodes an interleukin. An even more preferred cytokine nucleic acid molecule useful in the method of the present invention encodes interleukin-2 (IL-2), interleukin-7 (IL-7), interleukin-12 (IL-12), interleukin-15 (IL-15), interleukin-18 (IL-18), and/or ~~interferon-*~~ interferon-γ ~~(IFN*)~~ (IFN-γ). A most preferred cytokine nucleic acid molecule useful in the method of the present invention encodes interleukin-2 (IL-2), interleukin-12 (IL-12), interleukin-18 (IL-18) and/or ~~interferon-*~~ interferon-γ ~~(IFN*)~~ (IFN-γ).

Please replace paragraph [00119] with the following amended paragraph:

Transcription control sequences are sequences which control the initiation, elongation, and termination of transcription. Particularly important transcription control sequences are those which control transcription initiation, such as promoter, enhancer, operator and repressor sequences. Suitable transcription control sequences include any transcription control sequence that can function in at least one of the recombinant cells useful in the method of the present invention. A variety of such transcription control sequences are known to those skilled in the art. Preferred transcription control sequences include those which function in mammalian, bacteria, insect cells, and preferably in mammalian cells. More preferred transcription control sequences include, but are not limited to, simian virus 40

(SV-40), [[*-]] β-actin, retroviral long terminal repeat (LTR), Rous sarcoma virus (RSV), cytomegalovirus (CMV), *tac*, *lac*, *trp*, *trc*, oxy-pro, omp/lpp, rrnB, bacteriophage lambda [[(*)]] (γ) (such as [[*p_L]] γp_L and [[*p_R]] γp_R and fusions that include such promoters), bacteriophage T7, T7*lac*, bacteriophage T3, bacteriophage SP6, bacteriophage SP01, metallothionein, alpha mating factor, *Pichia* alcohol oxidase, alphavirus subgenomic promoters (such as Sindbis virus subgenomic promoters), baculovirus, *Heliothis zea* insect virus, vaccinia virus and other poxviruses, herpesvirus, and adenovirus transcription control sequences, as well as other sequences capable of controlling gene expression in eukaryotic cells. Additional suitable transcription control sequences include tissue-specific promoters and enhancers (e.g., T cell-specific enhancers and promoters). Transcription control sequences of the present invention can also include naturally occurring transcription control sequences naturally associated with a gene encoding an immunogen, including tumor antigen, an allergen, a pathogen antigen or a cytokine.

Please replace paragraph [00163] with the following amended paragraph:

A therapeutic composition of the present invention is particularly useful for eliciting an immune response in a mammal that has an infectious diseases caused by pathogens, including, but not limited to, bacteria (including intracellular bacteria which reside in host cells), viruses, parasites (including internal parasites), fungi (including pathogenic fungi) and endoparasites. Preferred infectious diseases to treat with a therapeutic composition of the present invention include chronic infectious diseases, and more preferably, pulmonary infectious diseases, such as tuberculosis. Particularly preferred infectious diseases to treat with a therapeutic composition of the present invention include human immunodeficiency virus (HIV), ~~Mycobacterium tuberculosis~~ *Mycobacterium tuberculosis*, herpesvirus, papillomavirus and ~~Candida~~ *Candida*.

Please replace paragraph [00171] with the following amended paragraph:

For antigen-specific immunization experiments, plasmid-based, eukaryotic expression vectors were utilized to express genes *in vivo*. Expression vectors (using pCR3.1, Invitrogen) for the cytokine cDNAs (IL-2, [[IFN*]] IFN-γ, IL-12) were all constructed using PCR amplification of RNA prepared from normal mouse spleens as described, for example in Sambrook et al., *supra*. The [[*- gal]] β-gal expression construct was provided by Dr. Cori Gorman. For immunization with these gene constructs, CLDC containing the desired gene constructs were injected by tail vein (i.e., intravenous delivery) or intraperitoneally (as

indicated) to deliver a total DNA amount of 5.0 to 10.0 [[ug]] μg DNA.

Please replace paragraph [00174] with the following amended paragraph:

Mice (3 per group, unless otherwise indicated) were injected intravenously or intraperitoneally, as indicated in the individual experiments, once with 100 ul of CLDC (prepared as described above) in D5W. Control mice were injected with 100 [[*l]] μL of D5W only. Three different strains of mice were evaluated in these experiments (C57Bl/6, BALB/c, ICR), but most of the data was generated using C57Bl/6 mice. The total amount of DNA injected was 10 μg per mouse, unless specified otherwise. At various time points post-injection (as indicated), the spleen and lung tissues were collected, mononuclear cell preparations were made, and the cells were assayed for expression of activation markers or cytokine release (see below).

Please replace paragraph [00176] with the following amended paragraph:

Upregulation of the early activation marker, CD69, which is upregulated on activated T cells, B cells, macrophages and NK cells, was used to assess early immune cell activation. Single cell suspensions were prepared from spleens of mice by NH_4Cl lysis procedure (Sambrook, *supra*), and lung mononuclear cells were prepared from lung tissues by collagenase digestion. Briefly, lung tissues were digested in 0.02% collagenase at 37°C for one hour. Lung mononuclear cells were purified from the digested tissue by Ficoll gradient centrifugation. For each experiment, spleen and lung cells were prepared from 3 animals per treatment group, unless noted otherwise. Cells were analyzed using a Becton-Dickinson FACSCalibur flow cytometer, with analysis gates set by first gating on spleen lymphocytes. Between 10,000 and 30,000 gated events were analyzed for each cell type. For analysis of cell activation, 3-color flow cytometric analysis was done, using anti-CD69 phycoerythrin (Pharmingen, San Diego, CA) to quantitate the number of CD69 positive cells. Cells were also dual-labeled to evaluate T cells (~~anti-**TCR~~ anti-αβ TCR antibody (biotin H57.597; Pharmingen) plus antibodies to either CD4 (FITC RM4-5; Pharmingen) or CD8 (FITC 53-6.7; Pharmingen). B cells were dual-labeled with anti--B220 (Pharmingen) and anti-IAb (FITC 3Fl2.35; provided by Dr. John Freed, National Jewish) or anti-IAd (FITC 14.44); NK cells were dual--labeled using anti NK 1.1 (biotin PK136; Pharmingen) and anti CD3 (FITC 2C11); macrophages were evaluated using anti-CR3 (biotin Mac-1; Pharmingen) and FITC anti-IAb or anti-IAd. The percentage of double positive cells expressing CD69 was determined for each cell type, and the mean (±SD) CD69+ cells plotted.

Please replace paragraph [00179] with the following amended paragraph:

Cytokine release was measured in spleen cell supernatants after either *in vivo* or *in vitro* stimulation. For assay of cytokine release after *in vivo* stimulation, spleen or lung mononuclear cells were prepared from mice either 6 or 24 hours after i.v. injection, then cultured at a concentration of 5 X 10^6 cells/ml for an additional 18 hours before supernatants were harvested. For *in vitro* stimulation of cytokine release, spleen cells were incubated *in vitro* with DNA, lipid, or DNA plus lipid at a final DNA concentration of 1.0 μg DNA per ml for 18 hours, at which time the supernatants were harvested for cytokine assay. Interferon--gamma [[(IFN*)]] (IFNγ) was assayed using a sandwich ELISA as is known in the art.

Please replace paragraph [00180] with the following amended paragraph:

The B16 (clone F1O) cells were obtained from Dr. Isiah Fidler (M D Anderson, Houston, TX); MCA-205 cells were provided by Dr Jack Routes (National Jewish); CT-26 cells were provided by Dr. Nicholas Restifo (National Cancer Institute); 4Tl cells were provided by Dr. Susan Rosenberg). All cell lines were maintained at 37°C in Modified Eagles medium supplemented with essential and non-essential amino acids, penicillin and glutamine, and 5% fetal bovine serum, and were treated periodically with ciprofloxacin (10 μg/ml) to maintain mycoplasma-free conditions. The [[*- gal]] β-gal transfected CT-26 tumor cell line (known as CL-25) was also provided by Dr. Nicholas Restifo.

Please replace paragraph [00183] with the following amended paragraph:

(a) The following experiment shows that intravenous (i.v.) injection of CLDC containing empty vector DNA induces marked activation of 5 different immune effector cell populations *in vivo*. In this experiment, CLDC were prepared which consisted of DOTAP and cholesterol mixed in a 1:1 molar ratio complexed with empty vector plasmid DNA (see Section A above). C57Bl/6 mice were injected intravenously with [[100 *l]] 100 μL of CLDC (10 μg empty vector DNA per mouse) in DW5 as described (Section C). 24 hours post-injection, spleen cells were harvested from control mice injected with diluent (D5W), and from mice injected with CLDC. Cells were labeled with specific antibodies to evaluate CD4+ and CD8+ T cells, NK cells, B cells, and macrophages and with an antibody to CD69 (early activation marker) and analyzed by flow cytometry (Section E). Figure 1 shows the

results from CD69/immune effector cell staining with control mice (open bars) and 3 CLDC-injected mice (black bars). Injection of CLDC (empty vector) induced pronounced upregulation of CD69 expression on all relevant immune effector cell populations, and similar results were observed as early as 6 hours post-administration (data not shown). These results indicate that systemic administration of CLDC (empty vector) induces massive and rapid immune activation.

Please replace paragraph [00190] with the following amended paragraph:

(h) The following experiment shows that cytokine release is induced by CLDC, but not by DNA or lipid alone. Spleen cells were incubated for 24 hours *in vitro* with CLDC (empty vector), DNA alone (empty vector), or lipid alone (DOTAP:cholesterol) and the supernatants were assayed for [[IFN*]] IFN-γ (as well as other cytokines, data not shown) (See Sections D and H). Figure 8 shows the results of an [[IFN*]] IFN-γ ELISA. As was observed for CD69 upregulation, cytokine release is also triggered only by the CLDC and not by either component alone. Thus, formation of the DNA-lipid complex clearly markedly accentuates any immune stimulatory properties that plasmid DNA and lipid alone might possess.

Please replace paragraph [00191] with the following amended paragraph:

(i) The following experiment demonstrates that injection of CLDC, but not poly I/C or LPS, induces [[IFN*]] IFN-γ production *in vivo*. C57Bl/6 mice (3 per group) were injected i.v. with 10 μg of either CLDC (empty vector), poly I/C, or LPS (as described in Sections A & C). Six hours later, spleen cells were harvested and cultured *in vitro* for an additional 12 hours. Then, cytokine levels in the supernatants were measured (Section H). Figure 9 shows that the *in vivo* cytokine response to CLDC injection was clearly different than the response to 2 other classical immune activating stimuli (LPS, poly I/C), thereby illustrating a marked difference between CLDC and other so-called non-specific immune stimulators.

Please replace paragraph [00192] with the following amended paragraph:

(j) The following experiment shows that NK cells are the source of [[IFN*]] IFN-γ production elicited by i.v. CLDC injection. To determine the cell type producing [[IFN*]] IFN-γ after injection of CLDC (empty vector), C57Bl/6 mice were depleted of NK cells using an anti-NK cell antibody (EV/aNK), or were untreated (control), or injected with CLDC and

untreated (EV/-) or injected with CLDC and treated with an irrelevant antiserum (EV/NRS) (as described in Section G). The amount of [[IFN*]] IFN-γ elaborated by spleen (Figure 10A) and lung cells (Figure 10B) 24 hours after injection of CLDC was quantitated (Section H). This experiment demonstrates that NK cells are the primary source of [[IFN*]] IFN-γ induced by i.v. administration of CLDC.

Please replace paragraph [00200] with the following amended paragraph:

The following experiment and Figures 17A-C show that intravenous injection of CLDC induces selective gene expression in pulmonary tissues. C57Bl/6 mice were injected i.v. with CLDC encoding a reporter gene, courteously provided by Dr. Robert Debs (luciferase; panel a), and the location of gene expression in various organs was determined 24 hours later (See Sections A, B and C). As shown in Figure 17A, luciferase gene expression was almost exclusively confined to pulmonary tissues. In Figures 17B and 17C, i.v. injection of CLDC encoding IL-2 or [[IFN*]] IFN-γ resulted in efficient intrapulmonary expression of IL-2 and [[IFN*]] IFN-γ, as demonstrated by determination of cytokine expression in lung tissues extracted from the mice. Injection of non-coding CLDC (EV) was included as an additional control.

Please replace paragraph [00201] with the following amended paragraph:

The following experiment and Figures 18A-F demonstrates that administration of cytokine genes using CLDC delivery improves the antitumor effect over empty vector alone. Using 3 different tumor models as described in Example 2 (MCA-205, Figures 18A and 18D; CT26, Figures 18B and 18E; B16, Figures 18C and 18F), we evaluated the antitumor effects of i.v. delivery of cytokine genes (IL-2, [[IFN*]] IFN-γ, and IL-12) using CLDC containing plasmid DNA expressing these genes, and compared the antitumor effects to those induced by empty vector DNA (See Sections A, B, C, and I). In both the day 3 treatment models (Figures 18A, 18B and 18C) and the day 6 treatment models (Figures 18D, 18E and 18F), addition of a cytokine gene that stimulates NK cells induced greater antitumor activity than the empty vector DNA alone, and this additional antitumor effect was particularly pronounced in the day 6 treatment models. It is believed that the added antitumor effect induced by the cytokine genes enhances and depends to a large degree on the initial immune activation inherent to administration of CLDC.

Please replace paragraph [00205] with the following amended paragraph:

(a) The following experiment shows that systemic immunization with CLDC encoding a tumor antigen induces strong antitumor activity *in vivo*. BALB/c mice (4 per group) were given 2.5 X 10^5 CL-25 tumor cells i.v. to establish pulmonary metastases (Section I). The CL-25 tumor line is derived from the CT26 colon carcinoma cell line and has been modified to express the [[*- gal]] β-gal antigen. Three days after administration of the CL-25 tumor cells, mice were treated with 2 i.v. administrations of CLDC encoding either nothing (EV) or the [[*- gal]] β-gal gene (B-gal), one week apart (Sections A, B, and C). One week after the second treatment, the mice were sacrificed and the antitumor effect was quantitated by counting the number of lung tumor nodules. Figure 20 shows that the number of tumors was significantly reduced by administration of empty vector CLDC (EV), but was even further reduced by administration of CLDC encoding the specific tumor antigen, ~~*-gal (B-gal)~~ β-gal. This experiment illustrates the principle that i.v. administration of CLDC encoding a tumor antigen (or antigen(s)) is an effective approach to eliciting immune responses against established tumors.

Please replace paragraph [00206] with the following amended paragraph:

(b) The following experiment demonstrates that i.v. administered CLDC-mediated immunization against a tumor antigen induces effective antitumor immunity, whereas intramuscular (IM) or intradermal (ID) immunization does not. Mice (4 per treatment group) with day 3 established CL25 lung tumors were treated by intravenous DNA immunization with [[*--]] β- gal DNA (Sections A, B, C, and I). Figure 21 shows that mice treated with intramuscular (B-gal/IM) or intradermal ~~(B-gal/ID)~~ β-gal/ID administration of 100 μg [[B-gal]] β-gal DNA showed no detectable antitumor effect as compared to control mice. By contrast, mice treated with [[*- gal]] β-gal CLDC (B-gal/IV; either 10 μg (10) or 1 μg (1) total DNA per mouse), had significantly reduced lung tumor burdens compared to control mice or to mice treated with i.v. administration of empty vector (EV/IV) CLDC, although i.v. administration of empty vector CLDC had a clear antitumor effect as compared to i.m. or i.d. administration of DNA. Thus, administration of 1/10th or 1/100th the amount of tumor antigen DNA using CLDC by i.v. administration was much more effective than conventional DNA immunization approaches.

Please replace paragraph [00207] with the following amended paragraph:

(c) The following experiment demonstrates that CLDC-mediated intravenous immunization with a tumor antigen induces an antigen-specific humoral response *in vivo*.

The relative efficiency of immunization via different routes of DNA administration was evaluated in BALB/c mice (4 per group) using plasmid DNA encoding the ~~*-galactosidase gene (*-gal)~~ β-galactosidase gene (β-gal). At 2 week intervals, serum was collected from each mouse and assayed for antibodies against the ~~*-gal~~ β-gal protein, using an antibody ELISA assay. Mice immunized by the intradermal and intramuscular route were injected once with 50 μg ~~*-gal~~ β-gal plasmid DNA. Mice immunized once by the intravenous route and intraperitoneal routes received 10 μg DNA that was complexed to a cationic liposome (CLDC). Control animals were not treated. The mean ~~*-gal~~ β-gal -specific antibody level (at a 1:1000 serum dilution) was determined for each group of mice and plotted for each of 4 different time points evaluated. Figure 22 shows that intravenous administration of CLDC containing 10 μg DNA elicited a similar antigen-specific humoral immune response to intradermal administration of 50 μg DNA, and both intravenous and intradermal administration elicited a more potent humoral immune response than either intraperitoneal or intramuscular injection of ~~*-gal~~ β-gal DNA.

Please replace paragraph [00208] with the following amended paragraph:

(d) The following experiment demonstrates that CLDC-mediated immunization with a tumor antigen induces antigen-specific production of [[IFN *]] IFN-γ by spleen cells. As another means of assessing the effectiveness of CLDC-mediated immunization, the release of [[IFN *]] IFN-γ (a cytokine with antitumor effects) was quantitated in spleen cells of mice that were immunized twice, one week apart, with either empty vector CLDC (EV), IL-2 CLDC (i.e., DNA encoding IL-2), or [[*- gal]] β-gal CLDC (DNA encoding [[*- gal]] β-gal) (Sections A, B, C & H). Figure 23 demonstrates that, mice immunized with the [[*-gal]] β-gal CLDC mounted a strong antigen specific immune response when re-challenged *in vitro* with the CL25 ([[*- gal]] β-gal transfected) cell line, as measured by [[IFN *]] IFN-γ production by splenocytes. In contrast, splenocytes from mice immunized with either empty vector CLDC (EV) or IL-2 CLDC (IL-2) produced very little [[IFN *]] IFN-γ. These data further substantiate the effectiveness of antigen-specific immunization using CLDC. It is believed that this effectiveness stems in large part from the innate immune response that is triggered by systemic administration of any CLDC. This strong induction of innate immune responses undoubtedly serves as a powerful adjuvant for inducing strong immune responses to the antigen-encoding DNA.

Please replace paragraph [00214] with the following amended paragraph:

(a) BALB/c mice (at least 8 per treatment group) were sensitized to ovalbumin as follows. Briefly, mice were sensitized by intraperitoneal (i.p.) injection of 20 μg ovalbumin (OVA) (Grade V, Sigma Chemical Co., St. Louis, MO) together with 20 mg alum (Al(OH)3) (Inject Alum; Pierce, Rockford, IL) in [[100 *l]] 100 μL PBS (phosphate-buffered saline), or with PBS alone. 72 hours before the mice were airway challenged with ovalbumin, the mice were treated with intravenous administration of [[IFN *]] IFN-γ CLDC (IFN-g) or empty vector CLDC (EV). Controls included OVA-sensitized mice that were not treated (IPN) as well as untreated mice that did not receive airway sensitization (IP). Mice received subsequent OVA aerosol challenge for 20 minutes with a 1% OVA/PBS solution. Airways responsiveness (Penh) following increasing doses of methacholine was assessed using whole body plethysmography (Buxco, Troy, NY) (asthma is known to increase the sensitivity of the airways to contractile agonists such as methacholine). In this system, an unrestrained spontaneously breathing mouse is placed into the main chamber of the plethysmograph, and pressure differences between this chamber and a reference chamber are recorded. The resulting box pressure signal is caused by volume and resultant pressure changes during the respiratory cycle of the animal. From these box pressure signals, the phases of the respiratory cycle, tidal volume, and the enhanced pause (Penh) can be calculated. Penh represents a function of the proportion of maximal expiratory to maximal inspiratory box pressure signals and of the timing of expiration. It correlates closely with pulmonary resistance measured by conventional two-chambered plethysmography in ventilated animals. Figure 27 shows that allergen sensitized and challenged mice which received intravenous administration of [[IFN*]] IFN-γ CLDC had significantly reduced airway hyperresponsiveness to methacholine challenge (i.e., almost equal to that of control (IP) mice), whereas airways responsiveness remained high in untreated animals (IPN). Animals treated with empty vector (CLDC) showed reduced hyperresponsiveness to methacholine at lower methacholine challenge doses. Additionally, both intravenous administration of [[IFN *]] IFN-γ CLDC and empty vector CLDC reduced airway hyperresponsiveness to methacholine significantly better than administration of recombinant [[IFN *]] IFN-γ protein (data not shown).

Please replace paragraph [00215] with the following amended paragraph:

(b) In this experiment, BALB/c mice were sensitized to ovalbumin as described in section (a) above, then treated with CLDC delivered either intravenously (IV) or intratracheally (IT). The degree of eosinophil infiltration into the airways (a measure of airways allergen sensitization) was quantitated in bronchoalveolar lavage fluid (BALF). The

mean number of eosinophils per ml BALF fluid was plotted for each group of mice (unsensitized control {IP}; sensitized, untreated control {IPN}; and sensitized mice treated with either intratracheal [[IFN *]] IFN-γ CLDC, intratracheal EV CLDC, intravenous [[IFN*]] IFN-γ CLDC, or intravenous EV CLDC). Figure 28 demonstrates that treatment with intravenous CLDC (both EV and [[IFN*]] IFN-γ CLDC) significantly reduced eosinophil infiltration compared to control (IPN) animals.

Please replace paragraph [00216] with the following amended paragraph:

The following example demonstrates that spleen and lung cells from mice receiving intravenous, but not intratracheal, administration of CLDC produce significant amounts of [[IFN*]] IFN-γ.

Please replace paragraph [00217] with the following amended paragraph:

BALB/c mice were administered CLDC containing 10 μg of DNA either intravenously or intratracheally as described in experiments above. 24 hours post-administration, [[IFN*]] IFN-γ production was measured from isolated spleen (Figure 29A) and lung (Figure 29B) cells of the animals. Figures 29A and 29B show that mice receiving intravenous administration of CLDC produced significant amounts of [[IFN*]] IFN-γ in contrast to mice receiving intratracheal administration of CLDC.

Please replace paragraph [00235] with the following amended paragraph:

13. The cytokine response to administration of CLDC is characteristic of the response to acute viral infections, and is dominated by release of [[IFN*]] IFN-γ from macrophages, NK cells, and other cell types throughout the body. This pattern of response is ideally suited for treatment of cancer, viral infections, and to serve as an adjuvant for certain types of vaccines.